5828

MOULD GROWTHS UPON COLD-STORE MEAT

13.

BY

HERB.

F. T. BROOKS, M.A. AND C. G. HANSFORD, B.A.

REPRINTED FROM THE
TRANSACTIONS OF THE BRITISH MYCOLOGICAL SOCIETY
VOL. VIII., PART III., 21 MARCH, 1923

IMPERIAL COLLEGE
OF
SCIENCE & TECHNOLOGY
BOTANY LIBRARY.

CAMBRIDGE
AT THE UNIVERSITY PRESS

[Reprinted from the
TRANSACTIONS OF THE BRITISH MYCOLOGICAL SOCIETY,
Vol. VIII, Part III, 1923.]

MOULD GROWTHS UPON COLD-STORE MEAT.

By F. T. Brooks, M.A. (University Lecturer in Botany, Cambridge, and Food Investigation Board), and C. G. Hansford, B.A. (Food Investigation Board).

CONTENTS.

		PAGE			PAGE
I.	Introduction . . .	113	VII.	*Saccharomyces* spp.	138
II.	*Cladosporium herbarum* .	116	VIII.	*Thamnidium* spp. .	138
III.	*Sporotrichum carnis* n.sp.	124	IX.	*Mucor* spp. . .	140
IV.	*Torula botryoides* n.sp. .	131	X.	Summary . .	140
V.	*Wardomyces anomala* n.sp.	135	XI.	References . .	141
VI.	*Penicillium* spp. . .	137			

I. INTRODUCTION.

An investigation of the mould fungi which contaminate chilled and frozen meat was commenced in 1918 when considerable quantities of imported meat were found to be damaged in this manner, probably chiefly on account of the abnormally long periods of storage which war conditions necessitated. Since then, numerous specimens of mould growths upon cold-store meat have been examined, and investigations have been made concerning the conditions under which these moulds develop. In 1921 a report (3) upon the "Black Spot" of chilled and frozen meat was published (Special Report No. 6, Food Investigation Board), and the present account contains a description of the other moulds which have been encountered during the course of the work*. These researches have been carried out under the auspices of the Food Investigation Board of the Department of Scientific and Industrial Research.

With one exception, all the fungi described have been repeatedly observed during the last three years upon cold-store meat, and in view of the multiplicity of fungoid forms, it is somewhat remarkable that those which occur upon meat are so few in variety. Our observations agree well with those of Massee (12), but he described in detail only *Cladosporium herbarum*, the cause of "Black Spot." Some of the other forms he mentioned, e.g. *Penicillium glaucum*, *Mucor mucedo* and *Mucor racemosus* we have seen, but *Oospora carneola*, *Verticillium lateritium*, *Penicillium candidum* and *Phycomyces nitens* we have not seen, notwithstanding repeated search. Klein (10) referred

* We are indebted to Mrs M. N. Kidd for assistance during the earlier stages of this investigation.

the cause of "Black Spot" to *Oidium carnis* but this is doubtless another, but incorrect, name for *Cladosporium herbarum*. Tabor (17) has recently contended that "Black Spot" of meat may be produced by many different fungi, but in our experience there is no satisfactory evidence for this statement. The fact that he has invariably failed to obtain growth from "Black Spots" shows that his methods are imperfect, and his contention that the "Black Spots" are due to the death of various mould fungi in the meat cannot be substantiated. In practically every case that came under our observation, the "Black Spots" on meat contained living mycelium which gave rise, under suitable conditions, invariably to *Cladosporium herbarum*, and to no other fungus. Furthermore, the only fungus with which we have been able to reproduce "Black Spot" on meat in cold storage is *Cladosporium herbarum*. It often happens that other mould growths of a more superficial character, such as *Penicillium* and *Mucor*, are superimposed upon "Black Spot," but in no case have they had anything to do with the very well-defined trouble of "Black Spot."

While this work was in progress, Monvoisin (13) in France, was making a similar investigation unknown to us. He published a short paper, the results of which agree in the main with our observations. The fungi he specifically mentions as occurring on cold-store meat are *Thamnidium elegans*, *Mucor mucedo*, *Rhizopus* sp. and *Penicillium glaucum*. At a much earlier date, Talayract (18) had recorded the occurrence of *Penicillium*, *Sporotrichum*, *Mucor* spp., *Dematium*, and pink yeasts upon imported meat which he had seen in the London docks, the *Dematium* mentioned here being doubtless *Cladosporium herbarum*.

Quite recently another French worker, Bidault (2), has published a brief account of the moulds of frozen meat. The forms of commonest occurrence according to him are much the same as those found by us, but in our experience *Botrytis* spp. and *Stysanus stemonitis* have not been seen. On the other hand, some species commonly found by us are not recorded by him. Details of experiments on the growth of these moulds at low temperatures are not given, but he states that *Chaetostylum Fresenii* (= *Thamnidium chaetocladioides*) and *Hormodendron cladosporioides* (= *Cladosporium herbarum*) will grow slightly at − 10° C., and that others will grow between − 6° and 0° C.

It was shown in the previous report (3) that the "Black Spot" fungus possessed the remarkable property of growth at − 6° C. Another of these meat moulds, *Torula botryoides*, was also found to develop at this low temperature, and more recently certain others have given evidence of slight power of development at − 6° C. All these forms grow readily at 0° to 2° C., and perhaps

others that have not yet shown growth at – 6° C. will be found capable of development between – 6° and o° C.; this will shortly be tested. Monvoisin(13) states that mould spores (species not stated) will not germinate under cold-storage conditions, but that these forms, if allowed to germinate for sixteen hours at ordinary temperature, will continue their growth in the cold-store and produce new sporing bodies in the course of four to five months. Monvoisin does not state definitely the forms with which he experimented, but he implies that they were the species mentioned above. Our own results show that the spores of *Cladosporium herbarum* germinate even at – 6° C., but that subsequent growth is more rapid if germination has taken place for a short period at ordinary temperature. Failure to get some of the other species to develop at – 6° C. may perhaps be due to the conditions of humidity being different from those in Monvoisin's experiments. There is no doubt that some of these mould contaminations are due to the meat being exposed to temperatures above o° C., especially a few degrees higher than this, as at more enhanced temperatures bacterial growth is so vigorous that mould development is inhibited to a great extent. On the other hand, it has been shown that prolonged storage may induce the formation of certain of these mould growths, e.g. "Black Spot," even at several degrees below o° C. Shorter storage at a temperature just below o° C. will also give opportunity for the development of some of these moulds.

The spores of all mould fungi found on cold-store meat retain their vitality for long periods, several, notably *Cladosporium herbarum, Penicillium expansum* and *Thamnidium* spp., remaining alive after being subjected to a temperature of – 6° C. for two years. A period of three years at this temperature has, however, killed even these forms unless growth has already occurred in the cold store.

Other common mould fungi also retain their vitality for long periods at low temperatures. For instance, spores of *Botrytis cinerea, Aspergillus niger* and *Acrostalagmus cinnabarinus,* germinated after being kept for a year on the surface of culture media at – 6° C., but spores of *Cephalothecium roseum, Fusarium coeruleum* and *Rhizopus nigricans* were killed under these conditions. It is noteworthy that prolonged exposure to cold retards the rate of germination of mould spores, and that young mycelia are more quickly killed by low temperatures than are spores. Recent research has indicated that even thin-walled fungal spores retain their vitality for much longer periods than was formerly supposed, and the present results confirm this.

In the course of this work it has been necessary to undertake a systematic study of many strains of certain of these fungi

which occur upon meat and upon vegetable substrata, and the observations upon the differences between these closely related strains are recorded here.

Several of these fungi are of common occurrence as moulds upon different kinds of organic matter, e.g. *Cladosporium herbarum*, but three are apparently new to science. Some occur commonly as moulds upon vegetable debris within and in the vicinity of abattoirs, and it is likely that all so occur. The similarity of the types of mould occurring on meat imported from different countries is very striking, and point to the cosmopolitan distribution of these fungi.

II. *CLADOSPORIUM HERBARUM.*

This fungus has been shown to be the cause of the trouble known in the frozen meat trade as "Black Spot." Several strains isolated from different kinds of meat have been proved to possess the power of growth below freezing point. Strains of this fungus have also been isolated from other sources, chiefly vegetable substrata, and some of these are also able to grow at $-6°$ C. For comparison, cultures of various species of *Cladosporium* were obtained from the Centraalbureau voor Schimmelcultures, Amsterdam. These were:

C. herbarum (No. 43) *C. epiphyllum* (No. 44)
C. Aphidis (No. 42) *C. carpophilum* (No. 47),

also *C. butryi* and *C. cucumerinum*, neither of which could be induced to fructify. In this connection it is noteworthy that other forms occasionally degenerated in the course of the work when cultivated for several generations on meat extract-peptone-agar.

In a paper on the mould-growths of frozen meat, Monvoisin (13) makes no special mention of *Cladosporium*. Bidault (2) records both *Cladosporium herbarum* and *Hormodendron cladosporioides* on frozen meat, but, for reasons which will be given later, we consider these to be identical.

In connection with the identification of the fungus causing "Black Spot" of meat, it was necessary to examine critically many closely related forms of *Cladosporium*. One of the results of this investigation has been to show that many so-called species of *Cladosporium* are not really specifically distinct from *C. herbarum*, but are only slightly different strains of the same fungus.

It is not only on meat that *Cladosporium herbarum* causes black spots. In September 1921, one of the writers saw dead fronds of the seaweed *Laminaria digitata* covered with black spots which to the naked eye appeared indistinguishable from

the "Black Spot" of meat (Fig. 1). Upon isolation, it was found that the cause of the black spots on the seaweed was also *Cladosporium herbarum*. The texture of such a seaweed is not unlike that of the connective tissue of meat upon which black spots are most prone to develop, and, as in the latter, the discoloration is due to the dark hyphae of the fungus ramifying in the tissues.

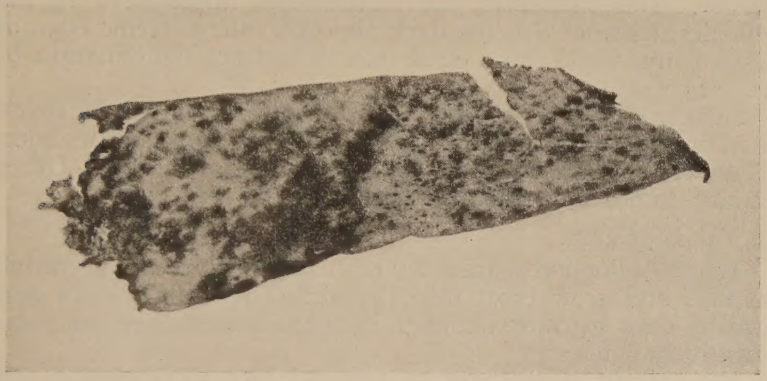


Fig. 1. "Black spot" on *Laminaria digitata*.

In view of growth proceeding at as low a temperature as − 6° C. it is clear that the sap of this fungus must possess a high osmotic concentration. The hyphae are narrow and it is difficult to observe plasmolytic phenomena in them, but immersion in a 15 % solution of calcium chloride undoubtedly causes plasmolysis while immersion in a 10 % solution is without effect.

The following is chiefly a systematic comparison of the characters of the different forms of *Cladosporium* isolated from meat and from vegetable sources.

I. Morphological characters, etc.

The following remarks apply to all strains used during this work. Each strain is denoted by a letter or number.

1. *Germination of Spores.*

The spores swell and then put out one, or sometimes two, germ tubes. These usually give rise to a branched mycelium, but occasionally when the conidium has given rise to two germ tubes, one of these forms a short conidiophore of the usual *Hormodendron* type.

2. *Hyphae.*

The hyphae are uniform in size and appearance in all strains, varying from 3–7 μ in diameter, and are septate at short intervals. In colour they vary from hyaline to almost black, according to age and strain. The length of time taken by the hyphae to turn dark olive varies with the strain, some, e.g. strain *S*, taking a very short time, others, e.g. *Z*, taking much longer. This influences the macroscopic appearance of the colonies, those of *S* being dark almost to the extreme edge of the colony, while those of *Z* show a distinct light margin of varying width.

Some strains, particularly *Z*, 60, 119A, when grown on Dox's medium, show fine, hyaline hyphae coiled in a peculiar manner. These arise as lateral branches of the normal hyphae. Other strains do not show this peculiarity.

3. *Conidiophores.*

The conidiophores arise as branches from the vegetative hyphae and grow erect into the air. They are septate and usually dark in colour, though in some strains they are comparatively light.

The present strains can be grouped into three classes, according to the length of the conidiophores, thus:

(*a*) Short conidiophores (less than 100 μ)—*Z*, 43, 108A, etc.
(*b*) Medium ,, (100–250 μ) —44.
(*c*) Long ,, (over 250 μ) —*S*, 110.

These classes are arbitrary and all gradations occur between the shortest and longest. This classification is based on a series of cultures in Petri dishes, on potato agar in the light at laboratory temperature. The width of the conidiophore is approximately that of the hyphae from which it arises.

The differentiation of the conidiophores from the vegetative mycelium varies in different strains. In *S*, the conidiophores are very distinct from the mycelium, whereas in *Z* the conidiophores are not differentiated. This distinction is more marked in those strains with long, unbranched conidiophores. In some strains the sterile part of the conidiophore is much branched, thus resembling the vegetative mycelium. In strain *S* the conidiophores are invariably unbranched, except at the apex where the head of conidia is formed.

4. *Formation of Conidia.*

The tip of the conidiophore is cut off by a wall to form the first conidium, this being invariably of the large type. The cell of the conidiophore immediately behind the first conidium may or may not grow out to form a second conidium, lateral to the

first. In nature each conidiophore usually bears a few large spores, but under culture conditions these large spores bud forth, giving rise to chains of smaller spores, the so-called *Hormodendron* stage. The youngest conidium is that at the distal end of the chain, the large conidia next the conidiophore being the oldest. Each conidium of a chain arises as a small bud on that immediately behind (cf. Fig. 2).

(1) 10 a.m. Mon. (2) 3.0 p.m. Mon. (3) 6 p.m. Mon. (4) 9.0 a.m. Tues.

Fig. 2. Formation of conidia of *Cladosporium herbarum*. (Hanging drop culture). × 900.

While still quite small, this bud is cut off by a membrane from the parent conidium, and continues to enlarge. The membrane between the young conidium and its parent is thickened from both sides simultaneously with the growth of the former, until finally, under certain conditions of microscope illumination, it appears as a small intercalary piece (cf. Fig. 3). On separation of the conidia, these "intercalary pieces" break asunder, along the line of the original membrane between the two conidia. Thus the majority of the conidia when separated, show a small stalk at each end, giving them a somewhat lemon-shaped appearance.

Fig. 3. Conidia of *C. herbarum* showing thickened end walls. × 3100.

The method of spore formation is the same in all the present series of forms.

5. *Conidia.*

As mentioned above, the conidia in artificial cultures differ considerably from those formed in nature. Upon vegetable debris in nature, the conidia are almost entirely of the large variety, while in cultures the large conidia are masked by the enormous numbers of small conidia formed by the process of budding described above. In culture, the conidia are in long branched chains, forming a more or less dense head at the end of the conidiophore. The number and length of these chains of small conidia is an index of the amount of budding that has taken place, and has a marked effect on the appearance of the conidiophore as seen intact under the microscope. In some strains, as *S*, the conidiophore terminates in a dense mass of chains of small conidia, while in other strains, as *Z*, the process of budding is much restricted, the conidiophore being terminated only by a small head of conidia. The budding influences the percentage of large conidia present, this being much greater in *Z* than in *S*.

The conidia vary greatly in size, from small spherical spores of about 4 μ in diameter, to large cylindrical conidia up to 25 $\mu \times$ 4–5 μ. The large basal conidia of the "head" merge gradually into the small spherical conidia of the distal parts of the chains. The large conidia are more or less cylindrical, tapering slightly at the ends, and may be 1–3 times septate, the septation being more frequent in old cultures.

According to Saccardo(15) and Rabenhorst(14) the large conidia of *C. herbarum* are constricted at the septa, but while this constriction is common in nature, though by no means universal, it is exceptional in artificial cultures, and is not characteristic of any particular strain. The same authors state that the walls of the conidia of a form (*Vincetoxici*) of *C. herbarum* are granular to echinulate, but in our cultures indications of a roughness of the conidial walls were very rare, although the walls of the conidia originally formed in nature by some strains were certainly rough.

The colour of the conidia as seen under the microscope varies with their age, the largest (i.e. also the oldest) conidia being darker in colour than the small conidia of the distal ends of the chains. In some strains, as *Z*, the conidia never become very dark in colour, while in *S* they become almost black. Every intermediate between these two extremes is present.

A minor point of difference between the two extreme strains *S* and *Z* is that the latter never formed truly spherical spores, while in *S* the fourth or fifth conidium from the base of the "head" was practically spherical.

II. Macroscopic Characters of Strains.

Although the various isolations approach each other so as to be almost indistinguishable under the microscope, to the naked eye there are considerable differences between them as indicated in the table, p. 125. Few cultures of the fungus isolated during the present work were absolutely identical in appearance. The strains were grown on Dox's medium with the full amount of sugar (30 gms. per litre). This medium proved to be the best for differentiating between the various strains, the cultures being compared at the end of two months' growth. Growth on this medium was luxuriant, and two characters were used to differentiate the strains:

(1) Colour.
(2) Texture of colony—whether "woolly" or not.

The "woolly" texture of some strains is due to the aerial growth of sterile mycelium—the hyphae being often very fine and almost hyaline—forming a layer over the conidiophores. The characters of the various strains were much the same when grown upon steamed potato.

III. Physiological Characters of Strains.

1. *Influence of Temperature.*

The approximate range of temperature within which each strain would grow was determined. The different strains show considerable divergence in this respect, and the grouping of the strains, according to this character, does not follow the arrangement according to other characters; the table given below is an attempt to classify them according to both.

As already recorded, some strains proved capable of growth below freezing point, at − 6° C., and probably others which would not grow at this temperature could develop at a slightly higher temperature, as all the strains isolated from "Black Spot" on meat in cold storage grew well at 2° C. Temporary freezing up to a month at − 6° C. had no effect on the rate or percentage of germination of the spores subsequently tested at room temperature, but prolonged freezing at − 6° C. eventually killed the spores of most strains, except such as proved capable of germination and growth at this temperature.

Temporary freezing (up to three weeks) of the vegetative mycelium of strain *S* had no permanently adverse effect, beyond greatly retarding growth.

2. *Influence of Light.*

With a view to the possibility of distinguishing forms of *Cladosporium* from those of *Hormodendron*, according to the

method of Schostakowitsch (16), a series of cultures was made in Petri dishes and exposed to illumination from one side only. A few strains showed a positive reaction to light, the conidiophores bending towards the source of light. The strains showing this characteristic were *S*, 102D, 103B, 113, all of which were originally isolated from meat in cold storage. Several other strains isolated from similar sources showed no reaction to light.

3. *Influence of increased concentration of mineral salts.*

Schostakowitsch (16) states that he was able to distinguish the two forms *Hormodendron* and *Cladosporium* by their characters when grown in solutions containing a high concentration of potassium nitrate. A series of cultures of the two strains *S* and 43 was made in a nutritive solution, to which various amounts of KNO_3 were added. Both strains grew in all strengths of KNO_3 up to saturation, and the differences between the two strains were much less in the more concentrated solutions than under more normal cultural conditions.

IV. Relation of *Cladosporium* to *Hormodendron*.

According to Bancroft (1) conidia of the *Cladosporium* type when germinated at a low temperature (below 56° F.), form a mycelium which produces other conidia of the same type. If germinated at a higher temperature (60° F. or above) he states that the conidia formed belong to the *Hormodendron* type. In the course of the present work it was found that Bancroft's statements could not be confirmed for any of the strains used. The "*Hormodendron*" stage was produced in every culture grown at low as well as at high temperatures on both solid and liquid media. Even at − 6° C. "Black Spot" produced by artificial inoculation of meat gave rise to many *Hormodendron* spores, although the proportion of large spores of *Cladosporium* type was greater than at ordinary temperature.

On examination of the present series of cultures it was found that the distinction between *Cladosporium* and "*Hormodendron*" was merely a question of the amount and character of the budding of the first-formed conidia as described above, and that the two forms merged gradually one into the other.

It is noteworthy that in nature the "*Hormodendron*" type is less common than the *Cladosporium*. In our experience the strains isolated from the latter type do not differ more from those isolated from the former type, than they differ among themselves. Thus the claim of Schostakowitsch (16) that he was able to differentiate the two types by

(*a*) the positive heliotropism of *Hormodendron*,

(*b*) the rough conidial walls of *Cladosporium*,
(*c*) the production of conidia by *Hormodendron* in a higher concentration of KNO_3 than *Cladosporium*,

is not supported by the present work.

No indication of any connection of *Cladosporium herbarum* with *Dematium pullulans* as mentioned by Delacroix and Maublanc (4) was observed in the course of this work, neither was there any evidence of a perithecial form as described by Janczewski (7-9).

V. Discussion.

Lindau in Rabenhorst's *Kryptogamen-Flora* in a note on the genus *Cladosporium*, mentions the great range of the species *C. herbarum*, and the consequent difficulty in diagnosing both this species and the whole genus. In a note on the "species" *C. epiphyllum* he expresses a doubt as to whether it is distinct from *C. herbarum*, and says that the two are often confused.

In the present work it was impossible to distinguish the Dutch culture of *C. epiphyllum* from some of the other forms. The whole of the present series of forms with the exception of No. 47 (*C. carpophilum* from Holland) were so closely allied in cultural and microscopic characters that separation into distinct species was impossible.

In Rabenhorst's *Kryptogamen-Flora* and also in Saccardo's *Sylloge*, the various "species" of *Cladosporium* are grouped more or less according to the substratum upon which they occur. This is very unsatisfactory, especially in view of the enormous range of hosts of the single species *C. herbarum*, and it is more than probable, in the light of the present work, that the great majority of the "species" described in these works ought to be included in *C. herbarum*. The diagnoses of many of the species are so vague that they cannot be taken seriously into consideration. Certainly, as a result of the present work, the two "species" *C. epiphyllum* and *C. Aphidis* must be included as synonyms of *C. herbarum*. These forms were sent from the Centraalbureau voor Schimmelcultures, Amsterdam, and represent, in our opinion, merely strains of the species *C. herbarum*.

On the other hand, the culture of *C. carpophilum* from the same source differed so greatly from all other strains that it must be retained as a distinct species. Thus, in view of the present work, the species of *Cladosporium* should be revised entirely, and diagnosed afresh, not only on the basis of their host plants and morphological characters as found in nature, but also upon their cultural characters.

VI. Table of differences between strains of *Cladosporium herbarum.*

The various strains included in the table on the opposite page are arranged as far as possible as intermediates between the two extremes, *S* and *Z*. Many other strains are not included, on account of their extreme "woolliness" on all media, which rendered it difficult to group them in this series. The table illustrates the wide range of the single species *C. herbarum*. The classification is based upon the following points, which were selected as the most definite criteria:

(1) colour and texture of colonies on Dox's medium,
(2) ,, ,, ,, steamed potato,
(3) length of conidiophores,
(4) branching of "stalk" of conidiophore,
(5) whether "heads" of conidia are dense or not (i.e. an index of the relative amount of budding of conidia),
(6) temperature relations,
(7) reaction to light.

III. *Sporotrichum carnis* n.sp.

This fungus was more frequently found upon all kinds of meat in cold storage than any other. It occurs in the form of innumerable white, slightly woolly patches, small in extent, and is the commonest form of "white mould" known to the meat trade. The growth of this fungus on meat is entirely superficial. It was present to some extent at any rate upon practically every sample of mouldy meat examined, although "Black Spot" caused by *Cladosporium herbarum* was sometimes more abundant. Talayract(18) mentions the occurrence of *Sporotrichum* upon chilled meat, but does not say what the species was.

Many slightly-differing strains were isolated from contaminated meat, but it is considered that all these belong to one species, a new one, *Sporotrichum carnis*. For comparison, cultures of *S. bombycinum* and *S. globuliferum* were obtained from the Centraalbureau voor Schimmelcultures, Amsterdam.

A form of *Sporotrichum*, indistinguishable from *S. carnis*, has recently been found to be of fairly common occurrence in slime fluxes of trees by Mr L. Ogilvy working at Cambridge, and one of us working in a Danish laboratory a short time ago encountered this fungus as a laboratory contamination.

Table of differences between strains of Cladosporium herbarum.

Strain	Origin	Macroscopic appearance on *Dox's medium* (two months' growth)	Length of Conidiophores	"Heads" of Conidia	Branching of Conidiophores	Range of temperature within which growth occurs*	Macroscopic appearance on potato chunks	Heliotropism
S	Meat in cold storage	Green brown, not woolly	Long	Very dense	Not branched	−6° C. 20° C.	Almost black, not woolly	+heliotropic
103B	,, ,,	,, ,,	,,	,,	,,	−6° C. 20° C.	,, ,,	,,
113	,, ,,	,, ,,	,,	,,	,,	2° C. 20° C.	,, ,,	,,
50B	*C. herbarum*? from Miss Smith, South Kensington	,, ,,	Medium-long	,,	Occas. branched	2 C. 25° C.	,, ,,	Not heliotropic
102D	Meat in cold storage	,, ,,	Long	,,	Often branched	?	,, slightly woolly	+heliotropic
114B	,, ,,	,, ,,	Medium-long	,,	,,	−6° C. 25° C.	Grey-green, very woolly	Not heliotropic
112	,, ,,	Green brown, woolly	,, very long	,,	Branched	2° C. 20° C.	,, ,,	,,
110	,, ,,	Bright green, slightly woolly	Very long	Not as dense as *S*	,,	2° C. 20° C.	Almost black, slightly woolly	,,
31	Black Currant twig	Green-black, not woolly	Medium	Very dense	Much branched	2° C. 25° C.	Black, light margin, slightly woolly	,,
44	"*C. epiphyllum*" from Holland	Bright green, not woolly	,,	,,	Branched	2° C. 25° C.	Green, very woolly	,,
37	Honeydew in greenhouse	,, ,,	Short-medium	Not as dense as *S*	,,	2° C. 30° C.	Almost black, not woolly	,,
54	Tomato fruit	Green-black ,,	,,	Very dense	,,	?	Light green, very woolly	,,
42	"*C. aphidis*" from Holland	Green-grey, slightly woolly	,,	Lax	Occas. branched	2° C. 20° C.	Green-brown, slightly woolly	,,
43	"*C. herbarum*" from Holland	Green-brown ,,	Very short	,,	Not branched	2° C. 30° C.	,, not woolly	,,
55	Maize shoot	Green-brown, woolly	Short	,,	Occas. branched	2° C. 25° C.	Green, woolly	,,
62	Wheat spikelet	,, ,,	Medium	Dense	,,	?	Green-brown, slightly woolly	,,
60	Dead leaves	Light green-grey, woolly	Short	Lax	Not branched	2° C. 25° C.	Grey-brown, very woolly	,,
108A	Meat in cold storage	Green, very woolly	,,	,,	,,	2° C. 25° C.	,, slightly woolly	,,
119A	,, ,,	Black, light margin ,woolly	,,	,,	,,	?	Green, slightly woolly	,,
Z	,, ,,	Green-black, slightly woolly	,,	,,	,,	−6° C. 25° C.	Green-brown, not woolly	,,

* The lower temperature is not necessarily the minimum.

I. Microscopical Characters.

The following remarks apply to all strains of *Sporotrichum carnis* used in this work, except where otherwise stated. The various strains are denoted either by a letter or a number.

1. *Germination of the spores.*

The spores swell considerably, each putting out one or two germ tubes, which give rise to a branched mycelium.

2. *Hyphae.*

The hyphae are very narrow, about 1 μ in diameter, and invariably hyaline. On some agar media the hyphae inside the medium differ from the normal type, being rather wider, and distinctly vacuolate. In the normal hyphae the septa are very obscure, but in these submerged hyphae the septa are more prominent. On agar the hyphae are usually straight, giving off branches almost at right angles to the parent hyphae.

The few strains which grow at 30° C. form peculiar stromatic colonies, consisting of swollen hyphae, the cells being very short and almost spherical. There is no penetration of the medium at this temperature as there is at ordinary temperatures.

3. *Formation of conidia* (Fig. 4).

(*a*) *Sporotrichum carnis.* Aerial branches arise from the vegetative mycelium, 30–50 μ in length. On these, other short branches arise, often in twos and threes, which may branch again. The branches are cut off by septa from the parent hypha, and the distal parts of the whole system of branches segment into short, cylindrical cells, 3–5 μ long. These cells form conidia, the apical portion of each swelling considerably, and their walls, especially the transverse wall at the base, thicken. Those cells of the conidiophore and its branches which do not form conidia disorganise, leaving nothing but the walls, which are almost invisible. The conidia are easily detached, and on mounting a portion of an old colony, a mass of spores is seen, with only occasional portions of hyphal walls.

(*b*) *Sporotrichum globuliferum* (from Amsterdam). As shown in Fig. 5, the conidiophore of *Sporotrichum globuliferum* differs greatly in appearance from that of the species isolated from meat. Long, aerial branches arise from the vegetative mycelium, many remaining sterile and contributing to the woolly appearance of the colonies, while others develop small groups of conidia at their ends and on short lateral branches. The conidia are both terminal and lateral on short sterigmata, those at the apex of the conidiophore being the youngest. This method of spore formation is distinct from that of *Sporotrichum carnis.*

4. *Conidia.*

The conidia of *S. carnis* vary greatly in size and shape. The majority, owing to their peculiar method of formation, are

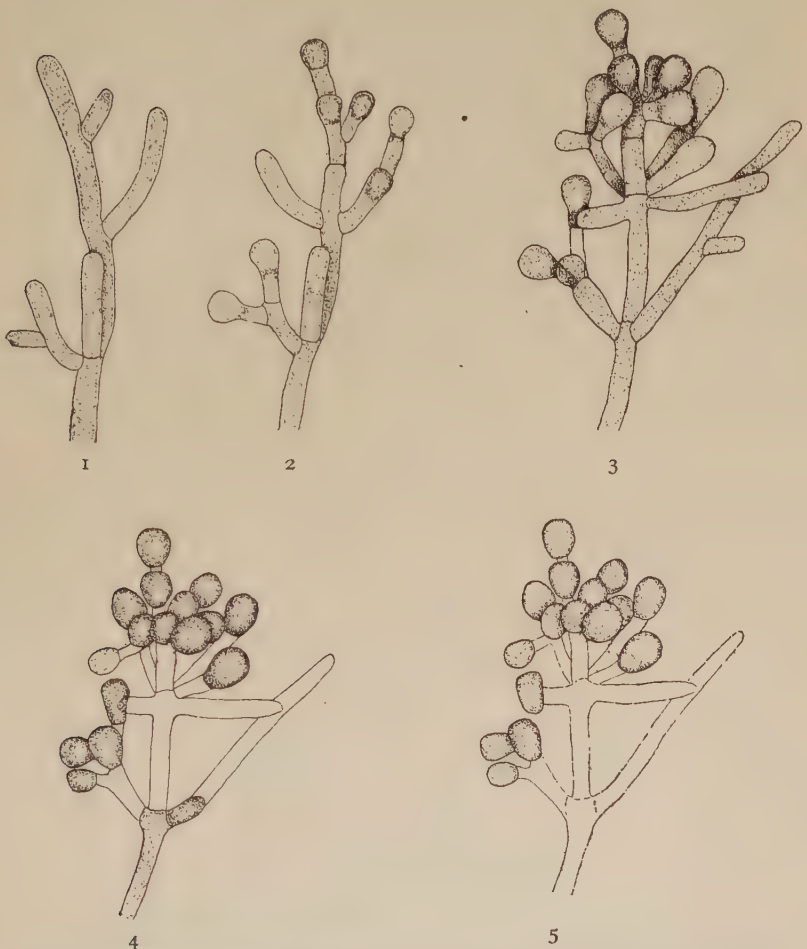


Fig. 4. Spore formation of *Sporotrichum carnis*. 1, young conidiophore from hanging drop culture, 48 hours old; 2, same, after a further 12 hours; 3, same, after a further 12 hours; 4, same as 3, 48 hours later; 5, same as 4, 48 hours later. × 2060.

somewhat pear-shaped, but others approximate to spherical. All the strains form hyaline conidia varying from 2–5 μ in length.

II. Macroscopic characters of strains of *S. carnis.*

The strains were cultivated on various media of which Dox's agar (containing half the usual amount of sugar), Sabouraud's glucose agar, and potato chunks were selected as best. On these media, especially on the second, the various strains, even when young, show marked differences. All are more or less white when young, but some show a peculiar yellow or orange discolouration inside the medium, which is absent from others. When the colonies are old, the aerial portions differ greatly in colour, varying in different strains from white to brown. Some strains are considerably more woolly in culture than others, especially on potato chunks.

III. Physiological characters (*S. carnis*).

(*a*) *Chemical.* The various isolations were grown in 4 % solutions of sugar to which peptone and litmus had been added.

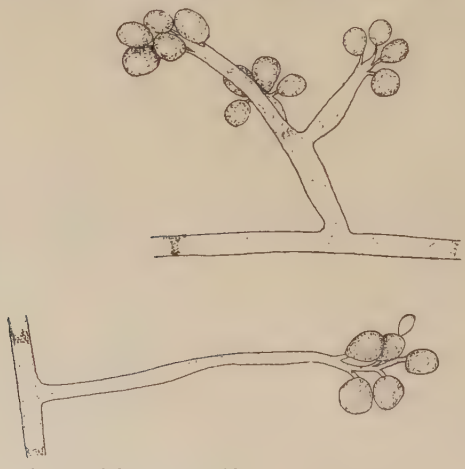


Fig. 5. *Sporotrichum globuliferum* conidiophores. × 2060.

They showed no great differences in their fermentation properties, all fermenting glucose, maltose, and laevulose, though the amount of acid produced varied to some extent. One or two forms also fermented sucrose. Some forms, especially those which showed a yellow discolouration of ordinary media, produced a yellow or orange pigment which diffused through the liquid. Three forms produced a dark-brown pigment instead of a yellow, and in one the litmus was bleached when the cultures were kept a month.

(*b*) *Temperature relations.* The forms were grown at different temperatures. Very slight indications of growth on artificial media at − 6° C. have been obtained in some strains after long periods, and as the majority of forms develop well at 2° C., it is likely that better growth occurs just below 0° C. than at − 6° C. The large white spots sometimes seen on meat have probably arisen through exposure to temperatures round about freezing point; there is no indication yet that such large growths would develop at − 6° C.

IV. Cultural differences of strains of *S. carnis.*

Scarcely any two isolations appeared identical in culture, but they can be arranged conveniently in four groups, according to their macroscopic appearance on Sabouraud's glucose agar, the medium on which they grow best. The microscopical characters of the strains are so similar as to be of no value for differentiating them.

1. *F Group*: comprising forms *F*, 121A, 104B, all isolated from meat in cold storage, and *L*, isolated from a Petri dish exposed in an abattoir in the Argentine.

This group is distinguished by its rather smooth colonies, which are not heaped up on the surface of the medium as in the next group, and by the development of a yellow pigment on almost all media used.

2. *W Group*: comprising forms *W*, 102E, 120B, 110A, all isolated from meat in cold storage.

These are very closely related and are characterised by their colonies being usually heaped up on the surface of the medium. In colour they vary from white to brown, and they produce no yellow pigment in the medium, as in the preceding group. Instead, in some liquid cultures they produce a dark brown pigment, diffusing through the liquid.

3. *"Woolly" Group*: comprising 118D, 120C, 103A, 101A, 104A, all isolated from meat in cold storage.

The extreme "woolliness" of these strains on all media distinguishes them from the other groups. The colonies are dirty brown in colour. These strains produce no yellow pigment in the medium, but some produce the dark brown pigment noted under the preceding group.

4. 119C *Group*: comprising 119C and 115G, isolated from meat.

On all media used these strains have very dense, smooth colonies, quite different from other groups. The colonies show

alternating coloured and white zones, the colour varying from pink to buff. On some media 115G develops a dark red colour.

It may be mentioned that *Sporotrichum globuliferum* differs both microscopically and macroscopically from all strains of *S. carnis*. On all media the colonies were light yellow in colour and extremely woolly, and in liquid cultures this fungus developed a yellow or orange pigment, which diffused into the medium.

V. Discussion.

The forms isolated from meat, although falling into the above groups may yet be classified as one species, as under the microscope no differences can be seen which would justify separation into distinct species.

As far as one can determine from the meagre diagnoses of other species of *Sporotrichum* given in Rabenhorst's *Kryptogamen-Flora* and in Saccardo's *Sylloge*, the present species differs from all others previously described. The diagnoses of the species given in these books are often so inadequate that identification is impossible, and unless re-diagnosis is possible it would be better to discard them. Diagnosis of such species should be based, not only on the characters exhibited when growing on the original substratum, but also on cultural characters on standard media under controlled conditions.

VI. Comparison with other Sporotrichums spp.

The diagnosis of the genus in Rabenhorst's *Kryptogamen-Flora* is as follows:

Hyphae: forming a "turf," septate or not septate, creeping or decumbent, irregularly but never verticillately branched; branches usually branched again.

Conidiophores: hardly differentiated, at most very like the ordinary side branches.

Conidia: lateral or terminal on the hyphae or on small branches, usually very numerous, may or may not have well-developed sterigmata, oval or spherical, hyaline or slightly coloured, very small.

In a note on the genus, Lindau says that "only in a few cases are the conidiophores erect, thereby being distinguished from the side branches; the spores are usually produced on the creeping mycelium, which soon disappears. Then the colony consists of masses of spores and remains of hyphae. The spores are produced terminally but through growth of the hyphae soon become lateral; the spores are often situated on small protuberances of the hyphae."

This method of spore formation is much the same as that

described for *S. globuliferum*, but is very different from that of *S. carnis*.

To this method of spore formation given in Rabenhorst, must be added a second, that of the present species as described above.

The diagnosis of this new species is as follows:

Sporotrichum carnis n.sp.*

Forming circular colonies, white, closely adpressed to the substratum.

Hyphae creeping, interwoven, branched, septate, septa very obscure, hyaline, 1–2 μ wide.

Conidiophores not well differentiated, much branched, hyaline.

Conidia formed laterally or terminally from slightly swollen distal cells of branches of conidiophores, hyaline, 2–5 μ × 2–4 μ, oval-pyriform. The conidiophores soon disorganise after formation of conidia. In artificial culture the colonies may appear coloured, varying from pale yellow to dark reddish brown and may be compact or woolly.

Habitat on meat which has been kept in cold storage.

IV. *TORULA BOTRYOIDES*, n.sp.

This fungus was first isolated in May 1918 from a halibut which had been kept in cold storage. The fish was sent to one of the writers in connection with another enquiry then in progress; upon arrival it was apparently free from moulds. Within a few days, however, whitish fluffy growths began to appear upon it, especially around the mouth and gills, notwithstanding the fact that the fish was kept in a refrigerator below 0° C. except when being examined. The same fungus was subsequently isolated from beef, mutton, rabbits, and sausages which had been kept in cold storage. On meat it produced a greyish-white, rather woolly growth, quite distinct in appearance from *Sporotrichum carnis*.

I. Microscopic characters.

1. *Germination of spores.*

The spores swell considerably and produce germ tubes, which give rise to a mycelium of branched hyphae. In this connection

* *Sporotrichum carnis* sp.nov.

Coloniis candidis substrato arcte adpressis; hyphis repentibus, intertextis, ramosis septatis (septis valde inconspicuis), hyalinis, 1–2 μ latis; conidiophoris haud bene evolutis, valde ramosis, hyalinis; conidiis in ramulorum tumidulis apicibus pleurogenis vel acrogenis, hyalinis, 2–5 μ × 2–4 μ, ovali-pyriformibus. Conidiophoris post conidia effecta mox dilabentibus. Coloniae in mediis nutrientibus cultae diverse coloratae, interdum pallide luteae interdum fusco-rubello-brunneae, et congestae vel flocculosae sunt.

Hab. Ad carnem in frigidariis asservatam.

We are indebted to Mr Gepp and Mr Ramsbottom of the British Museum for assistance in drawing up the Latin diagnoses.

it may be noted that no indication of the formation of a dense aggregation of cells was obtained, as described by Kr. Høye (6) for *Torula epizoa*, a common fungus on salted cod in Norway. Even when sown in fish extract with 10 % salt added, the spores germinated in the usual manner.

2. *Hyphae.*

The hyphae are hyaline to light olive in colour, septate at intervals, the septa being rather difficult to observe. The hyphae vary in width from 2–5 μ.

3. *Formation of spores.*

The "conidiophores" are very variable, and under certain conditions may be almost entirely absent. In all cultures the

Fig. 6. *Torula botryoides*, n.sp. *a*, conidiophore from culture in liquid fish extract, × 1500; *b*, conidiophore from culture on steamed potato showing method of spore-formation, × 2300.

spores are formed not only in dense heads on the conidiophores but also laterally in small groups of two or three along the ordinary hyphae. The "conidiophores" are never very distinct from the vegetative hyphae, and are usually much branched (Fig. 6). The ends of the branches of the conidiophores are more or less swollen, depending on the conditions of growth. On potato chunks at laboratory temperature the ends of the conidiophores form pronounced sterigmata, somewhat resembling those of *Penicillium*, and from which the conidia are abstricted, in long chains, as in *Penicillium*. On agar slopes at

2° C. the sterigmata are much less pronounced and the conidia appear to be borne in dense heads on the ends of the conidiophore. These apparent heads of conidia are made up of short chains, the base of each chain being attached to the conidiophore by a sterigma (Fig. 7).

Fig. 7. *Torula botryoides*, n.sp. Conidiophore from culture on agar at 2° C. × 600.

4. *Spores.*

The spores are spherical to oval in shape, 4–8 μ × 4–6 μ in size, the mean size of the conidia being very constant under all conditions of growth. When young they are hyaline, but become olivaceous when older. The chains of conidia easily break up into separate spores. The walls are smooth.

II. Macroscopic Characters.

The fungus does not spore at all well on potato agar at ordinary temperatures. The growth is chiefly vegetative, dark olive in colour and almost entirely within the medium. On other solid media, e.g. steamed carrot or potato, growth is more profuse and greyish in colour, and the colonies develop masses of spores. The various isolations are uniform in appearance under cultural conditions, showing a great contrast to those of *Sporotrichum carnis* and *Cladosporium herbarum.*

III. Physiological Characters.

The various isolations of this fungus were grown at temperatures of − 6, 2, 12, 25, 30° C. No growth took place at 25° or 30° C. At the ordinary laboratory temperature growth was fair but resulted in the formation of few spores upon potato agar, the cultures consisting almost entirely of mycelium within the agar slants. At 2° C. a copious aerial growth was produced upon the same medium, giving the culture a greyish-white appearance, whereas at laboratory temperature, the fungus was dull brown or black in colour. The fungus formed an abundance of spores on the agar cultures at 2° C., these being produced in groups of short chains on much branched conidiophores, as well as in isolated groups along the hyphae. Growth occurred at − 6° C., but was very slow, even slower than in *Cladosporium herbarum.*

IV. Discussion.

The systematic position of this fungus is obscure. On fish and meat, from which it was originally isolated, it was a greyish-white, woolly mould. In cultures on potato agar the colonies are brownish at ordinary temperature. In view of this, the fungus is considered to belong to the Dematiaceae, a conclusion supported by the formation of conidia which are hyaline to olivaceous in colour. The branched conidiophores with their sterigmata bearing spores, seem to be peculiar to this fungus, but for the present it is included in the genus *Torula.* The general appearance of the young conidiophores suggests the specific name *botryoides.* The diagnosis is as follows:

Torula botryoides n.sp.*

Colonies grey-white to fuscous, woolly, ¼–1″ in diameter. *Hyphae* septate, hyaline to light olive, 2–5 μ wide.

* *Torula botryoides* sp.nov.

Coloniis cinereo-albis vel fuscis, lanosis, 0·7–2·5 cm. diam., hyphis septatis hyalinis vel pallide olivascentibus, 2–5 μ latis. Conidiophoris e mycelio haud diversis, valde ramosis; conidiis in catenulis e sterigmatibus vel in acervulis

Conidiophores not distinct from the vegetative mycelium, much branched.

Conidia produced basipetally in long chains from sterigmata, also in groups of 1–3 along hyphae, hyaline to olivaceous, 4–8 μ × 4–6 μ, spherical to oval. On potato agar at ordinary temperatures colonies brown-black, and partly sterile. On steamed potato or carrot the colonies are grey-white and spore freely.

Habitat on fish and meat kept in cold storage.

Kr. Høye (6) describes a species of *Torula, T. epizoa,* upon dried salted cod in Norway, causing brown spots on the fish. Experiments showed that its growth was much restricted if no salt were present in the medium. Its growth was best in fish extract containing 10 % of salt. The colonies found on dried fish consisted mainly of a dense layer of chains of spores abstricted from the mycelium. The present fungus, *T. botryoides,* shows none of the above characters. On meat in cold storage the fungus forms greyish-white, rather fluffy patches, and its growth in cultures is much restricted if salt is added to the medium. Høye describes a peculiar cellular stroma developed by his fungus when grown in cod-extract gelatine containing less than 10 % salt, hyphae being very feebly developed. No such mode of germination has been observed in *Torula botryoides* under any conditions. It is clear that the two species are distinct.

W. G. Farlow (5) describes a fungus *Oidium* (*Torula*) *pulvinatum,* also found on the surface of dried cod in America, causing brown spots, but his diagnosis is not applicable to *T. botryoides,* as he states that the spores of *T. pulvinatum* are only 3–5·5 μ in diameter, and 12–15 in a chain, whereas the spores of *T. botryoides* are rather larger, and the chains may be much longer or absent. His drawing of a young conidiophore bears no resemblance to that of *T. botryoides. Oidium* (*Torula*) *pulvinatum* is probably identical with *Torula epizoa* but the diagnosis of these forms is inadequate.

V. *WARDOMYCES ANOMALA* n.gen. and n.sp.

This fungus was isolated once during the course of the investigation from a white, slightly woolly patch of mould on one of a consignment of skinned Australian rabbits which had been condemned on arrival here in consequence of serious contamina-

1–3nis e hypharum lateribus ortis, hyalinis vel olivascentibus, 4–8 μ × 4–6 μ, sphaericis vel ovalibus. Coloniae in agaro Solani tuberosi temperie normali cultae brunneo-atrae et partim steriles, in Solano cocto et in Dauco cinereo-albae et libere sporiferae sunt.

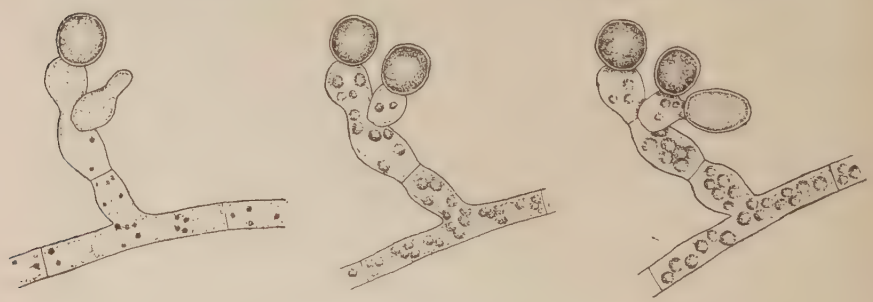
Hab. Ad carnem in frigidariis asservatam.

tion by mould growths. On the rabbit, this particular mould was almost indistinguishable from *Sporotrichum carnis*, but upon isolation it showed quite different microscopic characters.

The spores germinate to form a branched mycelium of hyaline hyphae 2–4 μ wide. The conidiophores arise as short, lateral branches, 15–25 μ long, from the vegetative hyphae; they remain hyaline and become septate with age. Some conidiophores are unbranched and form rarely short chains of two or three conidia, the distal conidium being the oldest; others branch repeatedly and form heads of spores which usually arise separately, although under certain cultural conditions two spores are formed occasionally in a chain. Two or three spores are often produced on each of the terminal cells of the branched conidiophore, one spore being formed terminally and the others in succession laterally (Figs. 8–10).

Fig. 8. Conidiophore of *Wardomyces anomala*, n.sp., from culture on agar. × 1500.

Fig. 9. Conidiophore of *Wardomyces anomala* n.sp., showing short spore chains. × 2300.



a *b* *c*

Fig. 10. Spore formation of *Wardomyces anomala*, n.sp. (*a*) Stage 1, from drop-culture 3 days old, × 1500. (*b*) Stage 2, 24 hours after stage 1, × 1500. (*c*) Stage 3, 24 hours after stage 2, × 1500.

The conidia are dark brown to black in colour, and vary in shape from sub-spherical to oval, with slightly pointed ends. They are 5–8 μ × 4–6 μ in size, with smooth walls which are almost opaque. The fungus grows well on the usual artificial media at ordinary temperature, but although isolated from rabbits which had been kept in cold storage does not grow at temperatures just above freezing point.

This fungus appears to be the type of a new genus of the Dematiaceae, the characteristic feature being the successive lateral proliferations of the basal cells of the conidiophores. The genus is named *Wardomyces* in memory of the late Prof. Marshall Ward.

Wardomyces n.gen.*

Mycelium creeping, septate, hyaline. Conidiophores formed as lateral branches of the mycelium, short, branched, septate, hyaline, the branches arising as successive lateral proliferations of the basal cells. Conidia abstricted singly, arising from the terminal cells of the conidiophores in lateral succession in groups, oval to spherical, brown to black.

The species diagnosis is as follows:

Wardomyces anomala n.sp.†

Colonies circular, white to fuscous, adpressed to the substratum, $\frac{1}{8}$–$\frac{1}{4}$" in diameter.

Hyphae creeping, branched, septate, 2–4 μ wide, hyaline.

Conidiophores short, 15–25 μ long, scarcely differentiated from the vegetative hyphae, branched or unbranched, hyaline.

Conidia abstricted singly, brown to black, smooth, subspherical to oval, usually with slightly pointed ends, 5–8 μ × 4–6 μ.

Habitat on flesh of rabbits kept in cold storage.

VI. *PENICILLIUM* spp.

Forms of the common blue-green mould were of frequent occurrence upon cold-store meat, and were occasionally found

* *Wardomyces* gen.nov.

Mycelio repente, septato, hyalino, conidiophoris e mycelio lateraliter exorientibus, brevibus, ramosis, septatis, hyalinis, ramis e cellulis basalibus successive emissis. Conidiis singillatim abstrictis, e cellulis conidiophorarum terminalibus successione laterali gregatim exorientibus, ovalibus vel sphaericis, e brunneo nigrescentibus.

† *Wardomyces anomala* sp.nov.

Coloniis circularibus, e albis fuscescentibus, substrato adpressis, 3–6 mm. diam., hyphis repentibus, ramosis, septatis, 2–4 μ latis, hyalinis. Conidiophoris brevibus, 15–25 μ longis, ab hyphis vegetativis vix differentibus, ramosis vel simplicibus, hyalinis. Conidio singillatim abstrictis, e brunneo nigrescentibus, laevibus, subsphaericis vel ovalibus, plerumque utrinque subacutis, 5–8 μ × 4–6 μ.

Hab. Ad cuniculorum carnem in frigidariis asservatam.

upon bacon also. When young, the colonies are white in colour, but when mature they are bluish green. The isolations proved generally to be *P. expansum*, but one has been provisionally identified by Dr Thom of Washington as *P. asperulum* Bainier.

Some evidence has been obtained that certain isolations of *P. expansum* from cold-store meat will germinate and grow at − 6° C., but development is very slow and after more than two years the colonies in the culture tubes or on meat are little more than just visible to the naked eye. Growth at this low temperature is sometimes more marked if germination of the spores has proceeded for 24–48 hours before the culture tubes are placed in the cold store. At − 1° to − 0·5° C. growth is more vigorous and at 2° C. it is active.

The spores and young mycelial growths also of this fungus are able to withstand exposure to a temperature of − 6° C. for long periods. Growth has sometimes taken place or has been resumed after $2\frac{1}{2}$ years at this temperature.

The large colonies of *Penicillium* seen on contaminated meat are in marked contrast to the small growths which have been produced under experimental cold storage conditions, and point to the fact that at some time or other the temperature of the meat has risen to about or just above freezing point. Spores of *Penicillium* are always present in the air, and doubtless are deposited upon the surface of the meat, ready to develop if conditions are suitable for growth. These bluish-green moulds are entirely superficial.

VII. *SACCHAROMYCES* spp.

Both white and pink yeasts were of common occurrence on meat contaminated with mould fungi. These forms develop with great rapidity at temperatures just above freezing point, but there is no evidence yet that they will grow below zero. The fungus described some years ago by Klein (10) as the cause of brown spots on chilled beef should, perhaps, be placed here. Yeast colonies in a dry condition are often brownish in colour, but it is not possible to be certain of the identity of the organism which Klein described. The white and pink yeasts isolated from meat do not form spores, and hence belong to the genus *Torula* used in the sense of Hansen and Jörgensen.

VIII. *THAMNIDIUM* spp.

Species of *Thamnidium* were frequently isolated from meat of various kinds in cold stores, which had become contaminated by mould growths, the type of which known in the meat trade as "whiskers" being usually due to species of this genus. Upon

meat these growths are profuse and are practically indistinguishable from species of *Mucor*. The genus *Thamnidium* differs from *Mucor* in the presence of two different kinds of sporangia, large and small, but in the natural occurrence of *Thamnidium* upon meat the small sporangia are either few or non-existent so that it appears like a *Mucor*. When grown under laboratory conditions, however, both types of sporangia are usually formed; upon nutrient agar small sporangia predominate, but upon cooked meat the reverse is the case. Upon fresh meat in the laboratory large sporangia sometimes occur to the exclusion of small ones.

Two species of *Thamnidium* were frequently isolated from cold-store meat, *T. elegans* and *T. chaetocladioides*, and of these the latter was the more common. It is of interest that these fungi are of rare occurrence upon other substrata, although upon mouldy meat they seem to be particularly common.

At ordinary temperatures and up to 20° C. these species grow well; at 25° C. *T. elegans* grows fairly well but *T. chaetocladioides* develops hardly at all, the spores becoming much swollen and forming curious amoeboid-looking cells. At 30° C. neither species develops.

So far there has been only the slightest signs of growth of either of these species at − 6° C., but at a temperature of 1–2° C. they grow profusely. They also grow at a temperature of − 1° to − 0·5° C.; development at temperatures between this and − 6° C. has not yet been tested. Bidault (2), however, states that *T. chaetocladioides* (= *Chaetostylum Fresenii*) grows at − 10° C.

The spores and even young mycelia will retain their vitality for long periods at a temperature of − 6° C. Thus meat inoculated with spores of *Thamnidium chaetocladioides* in October 1919 and placed in the cold store either immediately or after 24 hours, developed profuse growths of this fungus directly after removal to ordinary temperature in January 1921.

Mould spores of this type, therefore, which may have been deposited on the meat before being placed in store, remain living for long periods at low temperatures, and if there is a breakdown of the refrigerating plant, causing a rise in temperature to about freezing point, it is to be expected that these lurking moulds will develop profusely. Where such "whisker" growths are apparent on the meat, it is probable that the meat has been exposed to a temperature of 0° C. or slightly above for some time during storage. These moulds are entirely superficial and can readily be removed with a cloth. If unaccompanied by putrefactive bacteria, meat affected by these moulds is not dangerous for human consumption.

IX. *MUCOR* spp.

It was practically impossible to distinguish mould growths belonging to this genus from those of *Thamnidium* until the forms were isolated in culture. Together with *Thamnidium*, this genus is responsible for the profuse, greyish-white growths upon cold-store meat known as "whiskers," but species of *Mucor* are less frequently met with in this connection than are *Thamnidium elegans* and *T. chaetocladioides*.

Three species of *Mucor* were isolated from contaminated beef and mutton and these have been kindly identified by Prof. Lendner of Geneva as

M. mucedo Linné,
M. lusitanicus Bonderlein, and
M. racemosus Fres.

M. mucedo and *M. racemosus* are common moulds occurring upon a great variety of substrata.

Like *Thamnidium*, these fungi grow well at 1–2° C., but apart from slight germination of the spores of *M. mucedo* at – 6° C. there has been no indication of growth at this temperature. Tests have not yet been carried out between – 6° and 0° C. At temperatures just above zero, chlamydospores are formed more profusely than at ordinary temperatures.

M. mucedo and *M. lusitanicus* grew well and produced sporangia at all temperatures between 2° C. and 25° C., but did not grow at 30° C. *M. racemosus* grew at 30° C., but no sporangia were formed at that temperature.

The behaviour of these species of *Mucor* on cold-store meat is similar to that of *Thamnidium* and the presence of profuse growths of these forms is probably to be correlated with a rise in temperature to just above freezing point. Like *Thamnidium*, these species of *Mucor* do not penetrate the meat to any extent, nor do they confer poisonous properties upon it.

X. SUMMARY.

(1) The fungi which occur on cold-store meat coming to England from the southern hemisphere have been systematically examined. These moulds are: *Cladosporium herbarum* (the cause of meat "Black Spot"), *Thamnidium chaetocladioides*, *Thamnidium elegans*, *Mucor racemosus*, *Mucor mucedo*, *Mucor lusitanicus*, *Penicillium expansum*, *Penicillium anomalum*, *Saccharomyces* spp. together with two new species, *Sporotrichum carnis* and *Torula botryoides*, and the type species of a new genus, *Wardomyces anomala*.

(2) A general survey of many forms of *Cladosporium* has been undertaken, with the result that many so-called species of *Cladosporium* including *C. epiphyllum* are interpreted as strains

of *C. herbarum* and not distinct species. *Hormodendron cladosporioides* is a spore form of *C. herbarum*, and under cultural conditions is produced at low as well as at high temperatures.

(3) Some strains of *Cladosporium herbarum* will develop from spores at a temperature of − 6° C. and will give rise to considerable growths including conidiophores under prolonged cold-storage conditions. *Torula botryoides*, *Sporotrichum carnis*, *Penicillium expansum* and *Thamnidium* spp. sometimes develop slightly at this temperature, but readily at 0° C., and it is probable that they grow appreciably between these two temperatures; profuse growths of these forms on meat are usually an indication that the temperature has been raised to 0° C. or slightly higher at some time or other during storage. *Mucor* spp., *Saccharomyces* spp., and *Wardomyces anomala* do not develop at − 6° C., but will grow at 0° C. or just above.

(4) Spores and young mycelia of certain of these moulds, notably *Thamnidium* spp. and *Penicillium expansum*, retain their vitality for more than two years at − 6° C., and germinate or continue to develop on removal to ordinary temperatures.

(5) The growth of these moulds on meat is superficial, and even in "Black Spot" the mycelium penetrates only to a maximum depth of 4 mm. These fungi do not confer poisonous properties on the meat, and, unless associated with putrefactive bacteria, do not render the meat unfit for food.

(6) Several of these moulds are of common occurrence on vegetable debris and animal excreta, and their source is substrata of this nature occurring in and around abattoirs in the southern hemisphere. Air-borne spores alight upon the carcases before and during storage, and develop into mould growths at favourable opportunities.

(7) By controlling the temperature and humidity conditions in the cold stores, and by avoiding unduly prolonged storage, the growth of these fungi can be prevented.

XI. REFERENCES.

(1) Bancroft, K.—Researches on the life-history of parasitic fungi. Ann. Bot. xxiv, p. 359 (1910).

(2) Bidault, C.—Sur les moisissures des viandes congelées. Compt. rend. soc. biol. lxxxv (II), p. 1017 (1921).

(3) Brooks, F. T. and Kidd, M. N.—Black spot of chilled and frozen meat. Special report 6, Food Investigation Board, Dept. Sci. and Ind. Research, London, 1921.

(4) Delacroix, G. and Maublanc, A.—Maladies des plantes cultivées. Paris, 1909.

(5) Farlow, W. G.—Vegetable parasites of codfish. Bull. U.S. Fish Commission, vi, p. 1 (1886).

(6) Høye, Kr.—Undersølgelser over Klipfiskesoppen. Bergens Museums Aarsbog, vii, p. 40 (1901).

(7) Janczewski, E. von.—Polymorphisme du *Cladosporium herbarum*. Bull. Acad. Sci. de Cracovie, xxx, p. 417 (1892).

(8) Janczewski, E. von.—Les Périthéces du *Cladosporium herbarum*. Ibid. p. 271 (1893).
(9) —— Récherches sur le *Cladosporium herbarum*. Ibid. p. 147 (1894).
(10) Klein, E.—Report on the nature of brown spots on beef and on the nature of black spots on chilled beef. Quoted in Leighton and Douglas, "The meat industry and meat inspection," pp. 1544, 1553.
(11) Lafar, F.—Handb. d. Technisch. Mykol. iv, p. 270 (1906).
(12) Massee, G.—"Black Spot" of frozen beef. Journ. of Hygiene (1912).
(13) Monvoisin, M.—Les moisissures des viandes congelées. Rec. de Med. Veter. xciv (1918).
(14) Rabenhorst, L.—Kryptogamen-Flora.
(15) Saccardo, P. A.—Sylloge Fungorum.
(16) Schostakowitsch, W.—Über die Bedingungen der Conidienbildung bei Russthaupilzen. Flora, lxxxi, p. 362 (1895).
(17) Tabor, C. J.—Black spot on chilled and frozen meat. Cold Storage and Produce Review, Oct. 20th, 1921.
(18) Talayract, M. J.—Conserves de viandes par les procédés frigorifiques. Ann. d'hygiene publique, sér. 3, xlv, p. 166 (1901).